FIG. 1

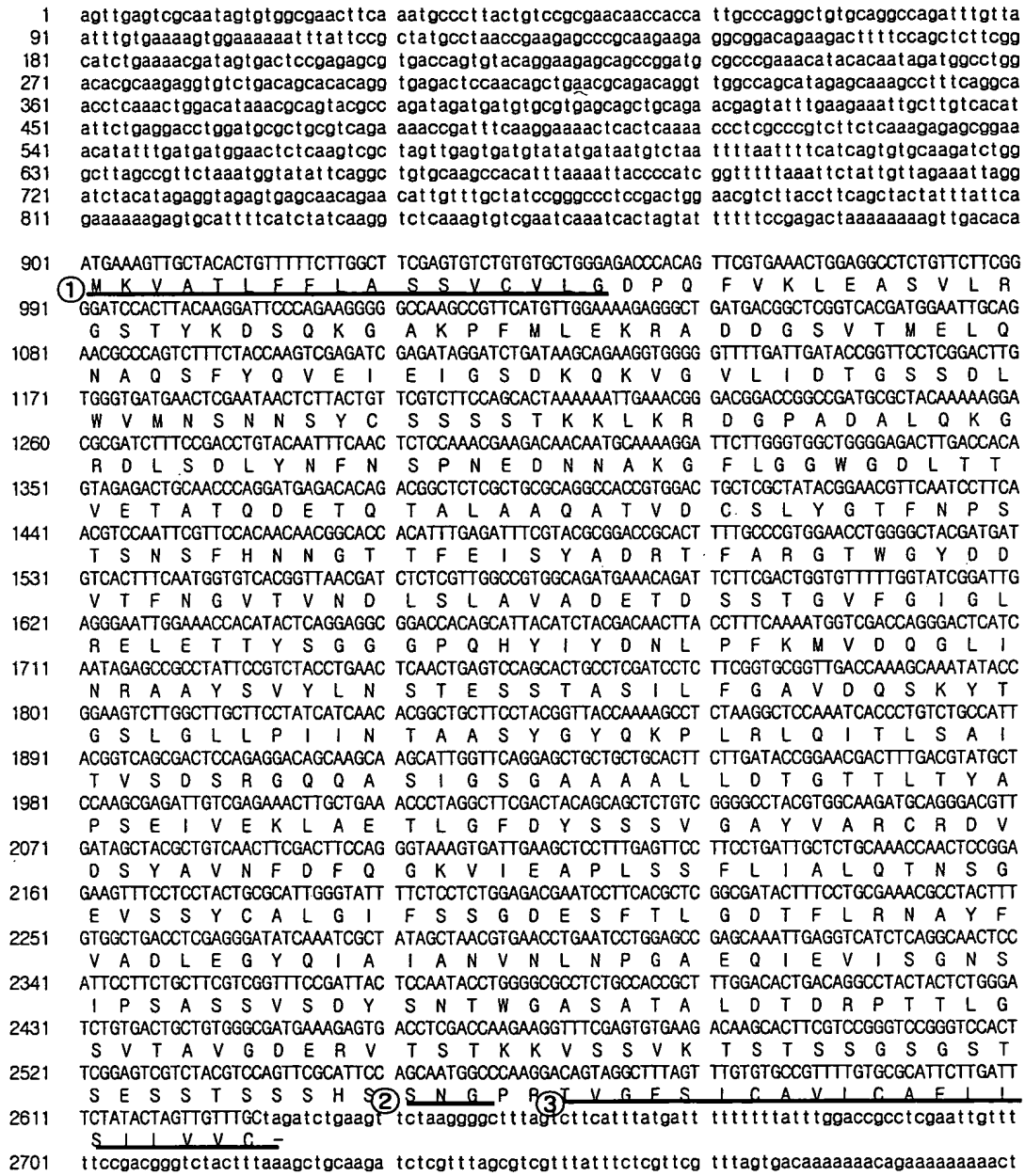


FIG. 2

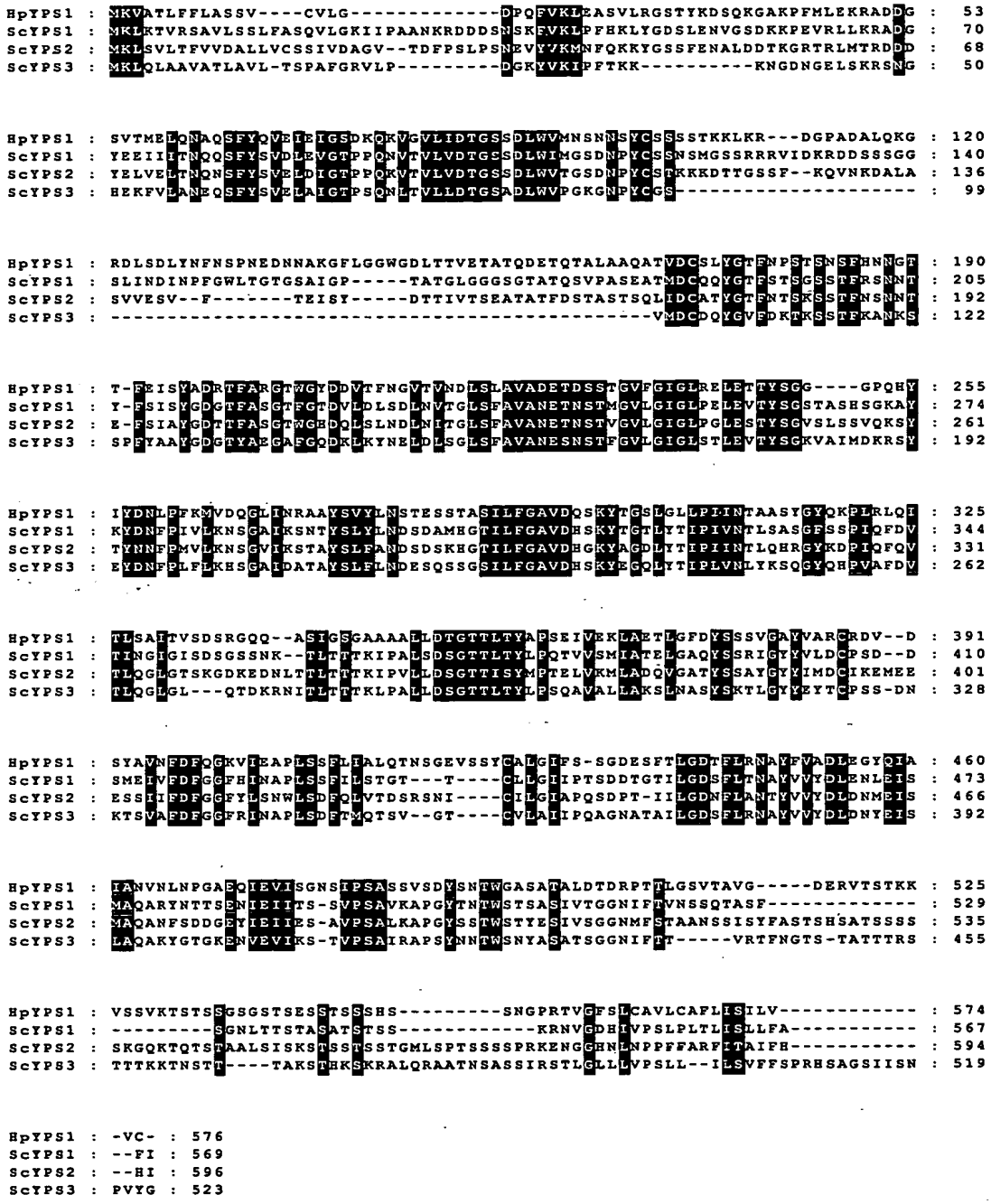

HpYPS1 : MKVATLFFLASSV----CVLG----------DPQFVKLEASVLRGSTYKDSQKGAKPFMLEKRADDG : 53
ScYPS1 : MKLKTVRSAVLSSLFASQVLGKIIPAANKRDDDSNSKFVKLPFEKLYGDSLENVGSDKKPEVRLLKRADG : 70
ScYPS2 : MKLSVLTFVVDALLVCSSIVDAGV--TDFPSLPSNEVYYKMNFQKKYGSSFENALDDTKGRTRLMTRDDD : 68
ScYPS3 : MKLQLAAVATLAVL-TSPAFGRVLP---------DGKYVKIPFTKK----------KNGDNGELSKRSNG : 50
HpYPS1 : SVTMELQNAQSFYQVEIEIGSDKQKVGVLIDTGSSDLWVMNSNNSYCSSSSTKKLKR---DGPADALQKG : 120
ScYPS1 : YEEIILTNQQSFYSVDLEVGTPPQNVTVLVDTGSSDLWIMGSDNPYCSSNSMGSSRRRVIDKRDDSSSGG : 140
ScYPS2 : YELVELTNQNSFYSVELDIGTPPQKVTVLVDTGSSDLWVTGSDNPYCSTKKKDTTGSSF--KQVNKDALA : 136
ScYPS3 : HEKFVLANEQSFYSVELAIGTPSQNLTVLLDTGSADLWVPGKGNPYCGS---------- : 99
HpYPS1 : RDLSDLYNFNSPNEDNNAKGFLGGWGDLTTVETATQDETQTALAAQATVDCSLYGTFNPSTSNSFHNNGT : 190
ScYPS1 : SLINDINPFGWLTGTGSAIGP-----TATGLGGGSGTATQSVPASEATMDCQQYGTFSTSGSSTFRSNNT : 205
ScYPS2 : SVVESV--F--------TEISY-----DTTIVTSEATATFDSTASTSQLIDCATYGTFNTSKSSTFNSNNT : 192
ScYPS3 : ----------VMDCDQYGVFDKTKSSTFKANKS : 122
HpYPS1 : T-FEISVADRTFARGTWGYDDVTFNGVTVNDLSLAVADETDSSTGVFGIGLRELETTYSGG-----GPQHY : 255
ScYPS1 : Y-FSISYGDGTFASGTFGTDVLDLSDLNVTGLSFAVANETNSTMGVLGIGLPELEVTYSGSTASHSGKAY : 274
ScYPS2 : E-FSIAYGDTTFASGTWGEDQLSLNDLNITGLSFAVANETNSTVGVLGIGLPGLESTYSGVSLSSVQKSY : 261
ScYPS3 : SPFYAAYGDGTYAEGAFGQDKLKYNELDLSGLSFAVANESNSTFGVLGIGLSTLEVTYSGKVAIMDKRSY : 192
HpYPS1 : IYDNLPFKMVDQGLINRAAYSVYLNSTESSTASILFGAVDQSKYTGSLGLLPIINTAASYGYQKPLRLQI : 325
ScYPS1 : KYDNFPIVLKNSGAIKSNTYSLYLNDSDAMHGTILFGAVDHSKYTGTLYTIPIVNTLSASGFSSPIQFDV : 344
ScYPS2 : TYNNFPMVLKNSGVIKSTAYSLFANDSDSKHGTILFGAVDEGKYAGDLYTIPIINTLQHRGYKDPIQFQV : 331
ScYPS3 : EYDNFPLFLKHSGAIDATAYSLFLNDESQSSGSILFGAVDHSKYEGQLYTIPLVNLYKSQGYQHPVAFDV : 262
HpYPS1 : TLSAITVSDSRGQQ--ASIGSGAAAALLDTGTTLTYAPSEIVEKLAETLGFDYSSSVGAYVARCRDV--D : 391
ScYPS1 : TINGIGISDSGSSNK--TLTTTKIPALSDSGTTLTYLPQTVVSMIATELGAQYSSRIGYYVLDCPSD--D : 410
ScYPS2 : TLQGLGTSKGDKEDNLTTLTTTKIPVLLDSGTTISYMPTELVKMLADQVGATYSSAYGYYIMDCIKEMEE : 401
ScYPS3 : TLQGLGL---QTDKRNITLTTTKLPALLDSGTTLTYLPSQAVALLAKSLNASYSKTLGYYEYTCPSS-DN : 328
HpYPS1 : SYAVNFDFQGKVIEAPLSSFLIALQTNSGEVSSYCALGIFS-SGDESFTLGDTFLRNAYFVADLEGYQIA : 460
ScYPS1 : SMEIVFDFGGFHINAPLSSFILSTGT---T----CLLGIIPTSDDTGTILGDSFLTNAYVVYDLENLEIS : 473
ScYPS2 : ESSIIFDFGGFYLSNWLSDFQLVTDSRSNI----CILGIAPQSDPT-IILGDNFLANTYVVYDLDNMEIS : 466
ScYPS3 : KTSVAFDFGGFRINAPLSDFTMQTSV--GT----CVLAIIPQAGNATAILGDSFLRNAYVVYDLDNYEIS : 392
HpYPS1 : IANVNLNPGAEQIEVISGNSIPSASSVSDVSNTWGASATALDTDRPTTLGSVTAVG-----DERVTSTKK : 525
ScYPS1 : MAQARYNTTSENIEIITS-SVPSAVKAPGYTNTWSTSASIVTGGNIFTVNSSQTASF---------- : 529
ScYPS2 : MAQANFSDDGEYIEIIES-AVPSALKAPGYSSTWSTYESIVSGGNMFSTAANSSISYFASTSHSATSSSS : 535
ScYPS3 : LAQAKYGTGKENVEVIKS-TVPSAIRAPSYNNTWSNYASATSGGNIFTT-----VRTFNGTS-TATTTRS : 455
HpYPS1 : VSSVKTSTSSGSGSTSESSTSSSHS----------SNGPRTVGFSLCAVLCAFLISILV---------- : 574
ScYPS1 : ----------SGNLTTSTASATSTSS----------KRNVGDHIVPSLPLTLISLLFA---------- : 567
ScYPS2 : SKGQKTQTSTAALSISKSTSSTSSTGMLSPTSSSSPRKENGGHNLNPPFFARFITAIFH---------- : 594
ScYPS3 : TTTKKTNSTT----TAKSTHKSKRALQRAATNSASSIRSTLGLLLVPSLL--ILSVFFSPRHSAGSIISN : 519
HpYPS1 : -VC- : 576
ScYPS1 : --FI : 569
ScYPS2 : --HI : 596
ScYPS3 : PVYG : 523

FIG. 3

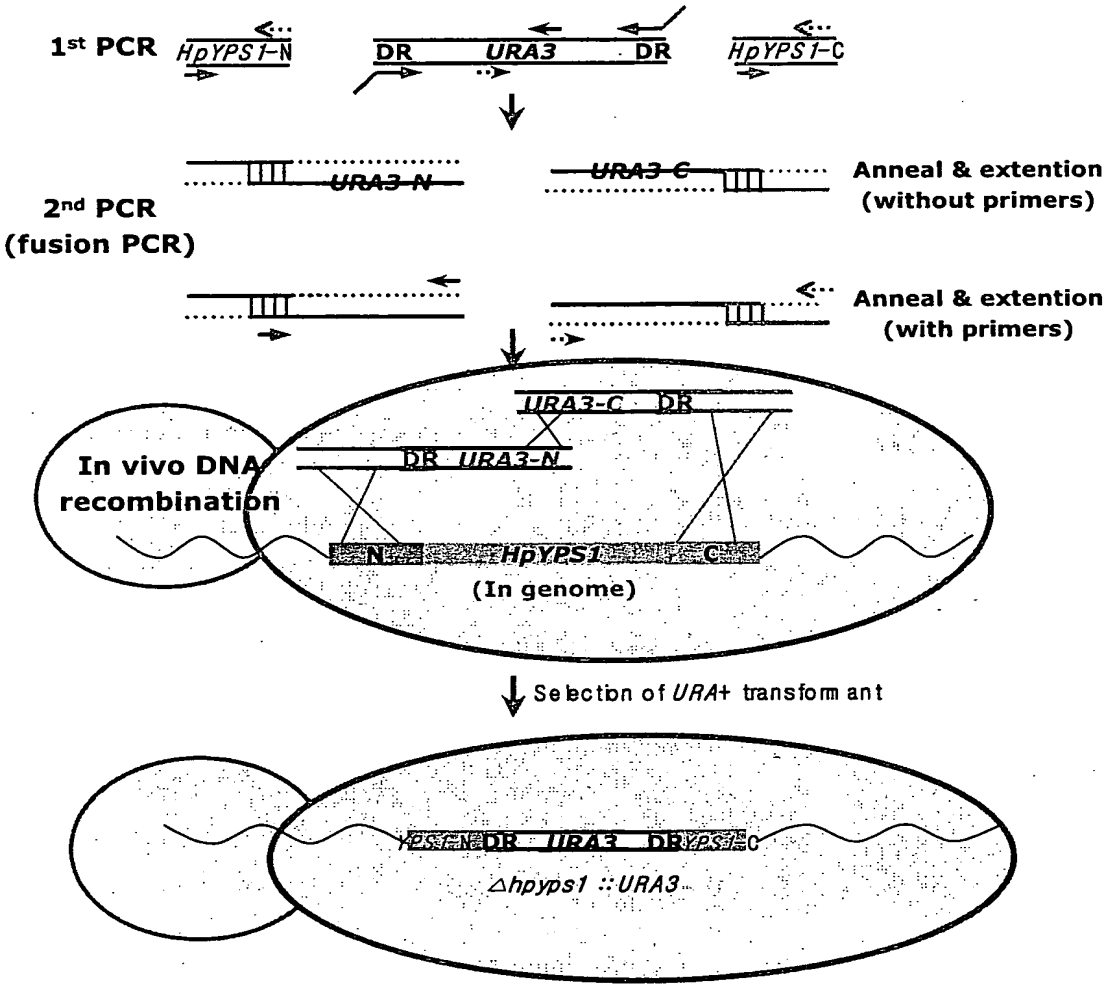

1st PCR
HpYPS1-N
DR
URA3
DR
HpYPS1-C
URA3-N
URA3-C
Anneal & extention
(without primers)
2nd PCR
(fusion PCR)
Anneal & extention
(with primers)
In vivo DNA
recombination
URA3-C
DR
DR
URA3-N
N
HpYPS1
C
(In genome)
Selection of URA+ transformant
DR
URA3
DR
C
△hpyps1 ::URA3

FIG. 4

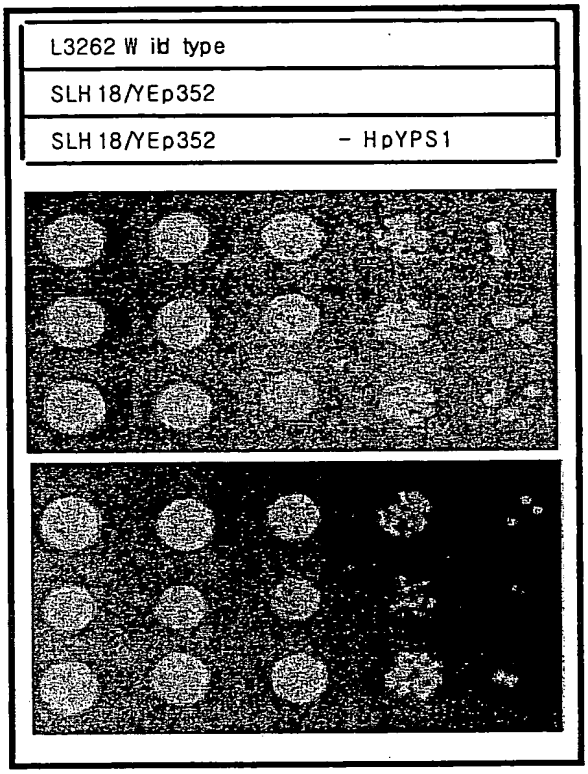
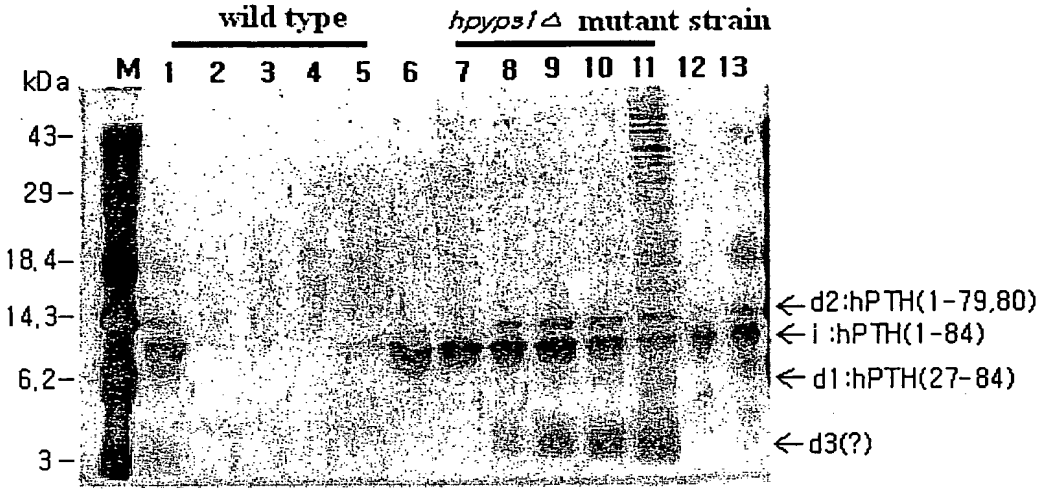

L3262 W ild type
SLH 18/YEp352
SLH 18/YEp352 - HpYPS1
30 ℃ , 48 hr
37 ℃ , 48 hr

FIG. 5



Lane M: Molecular marker
1: wild type, o-h reaction
2: wild type, 2-h reaction
3: wild type, 4-h reaction
4: wild type, 6-h reaction
5: wild type, 24-h reaction
6: distilled water + hPTH, o-h reaction
7: mutant strain, o-h reaction
8: mutant strain, 2-h reaction
9: mutant strain, 4-h reaction
10: mutant strain, 6-h reaction
11: mutant strain, 24-h reaction
12: hPTH 100 ng
13: hPTH 200 ng

6/10
FIG. 6

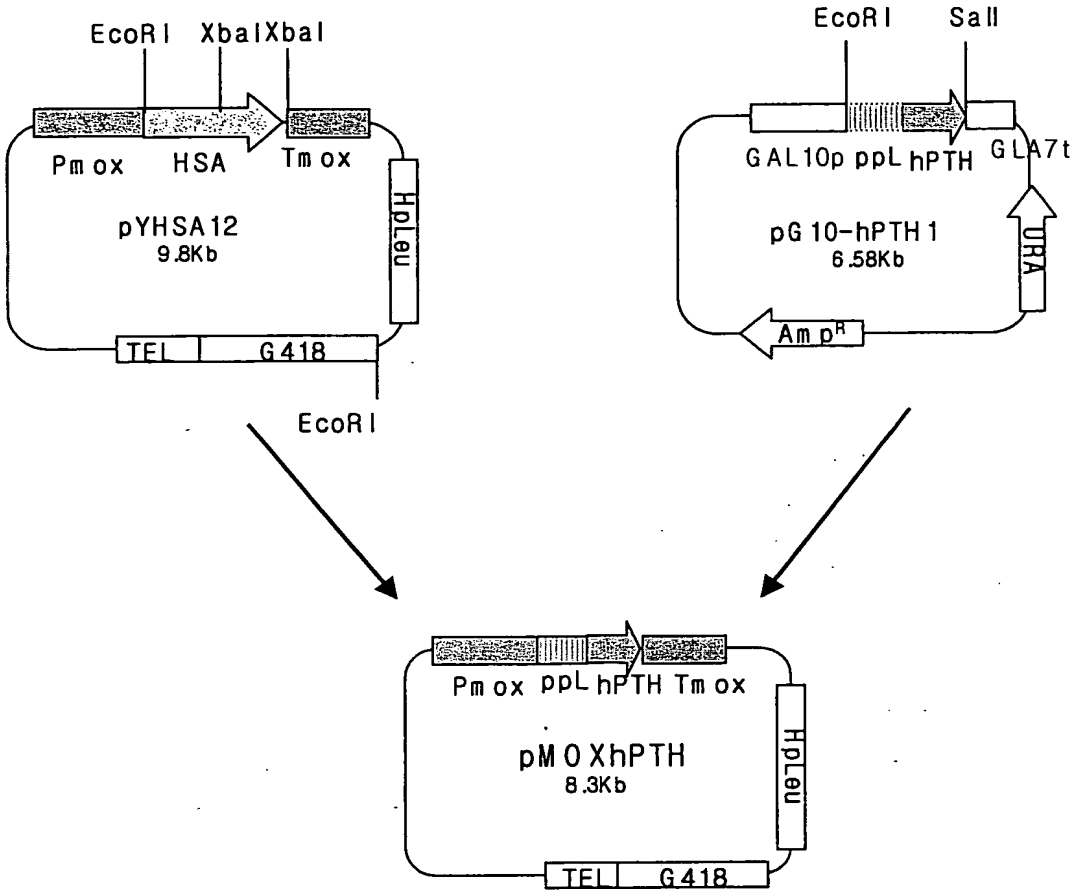
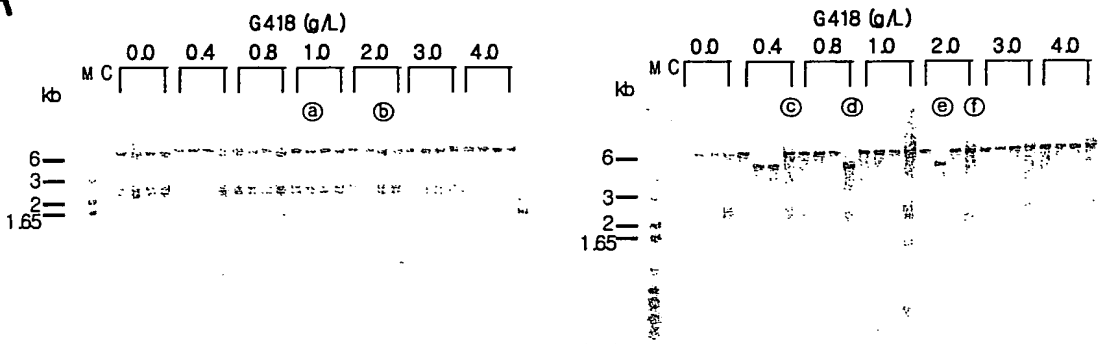

EcoRI
XbaI
XbaI
Pmox
HSA
Tmox
pYHSA12
9.8Kb
HpLeu
TEL
G418
EcoRI
EcoRI
SalI
GAL10p
ppL hPTH
GLA7t
pG10-hPTH1
6.58Kb
URA
AmpR
Pmox
ppL hPTH
Tmox
pM0XhPTH
8.3Kb
HpLeu
TEL
G418

FIG. 7

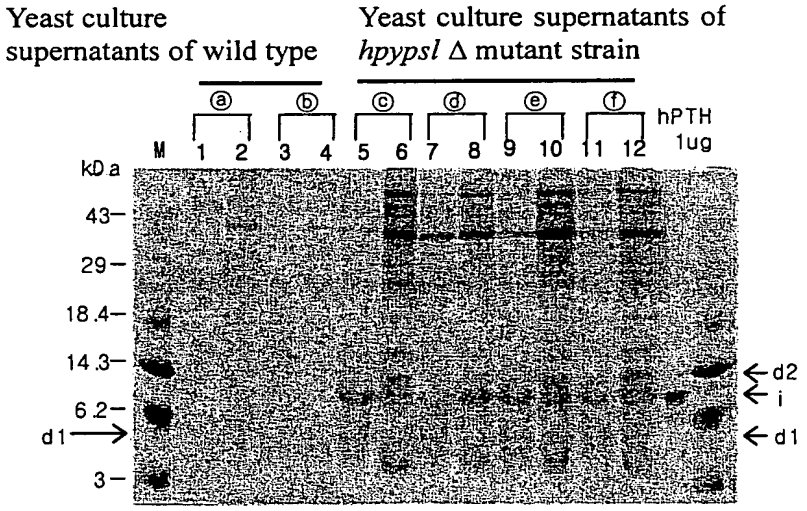
A



Transformants of wild type

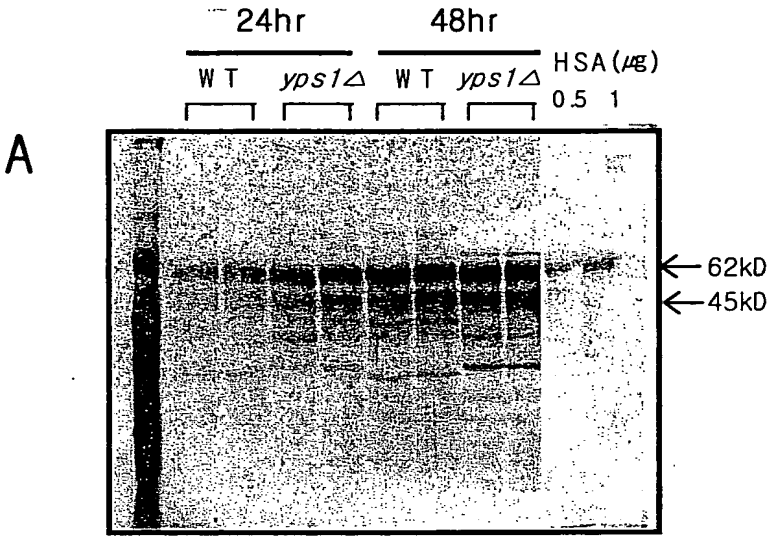
Transformants of *hpypsl* Δ mutant strain

B



Lane 1, 3, 5, 7, 9, 11 : 12hr after initiation of the cultivation
Lane 2, 4, 6, 8, 10, 12 : 24hr after initiation of the cultivation

FIG. 8


24hr
48hr
WT
ypsl△
WT
ypsl△
HSA(μg)
0.5 1
A
62kD
45kD

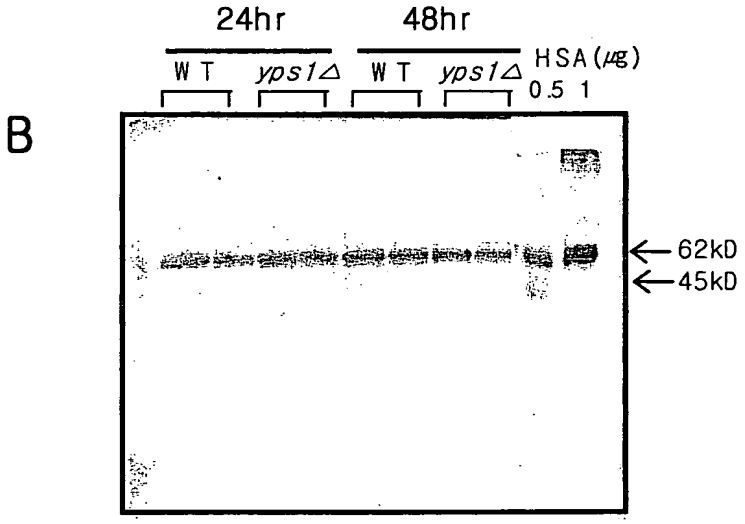

24hr
48hr
WT
ypsl△
WT
ypsl△
HSA(μg)
0.5 1
B
62kD
45kD

FIG. 9

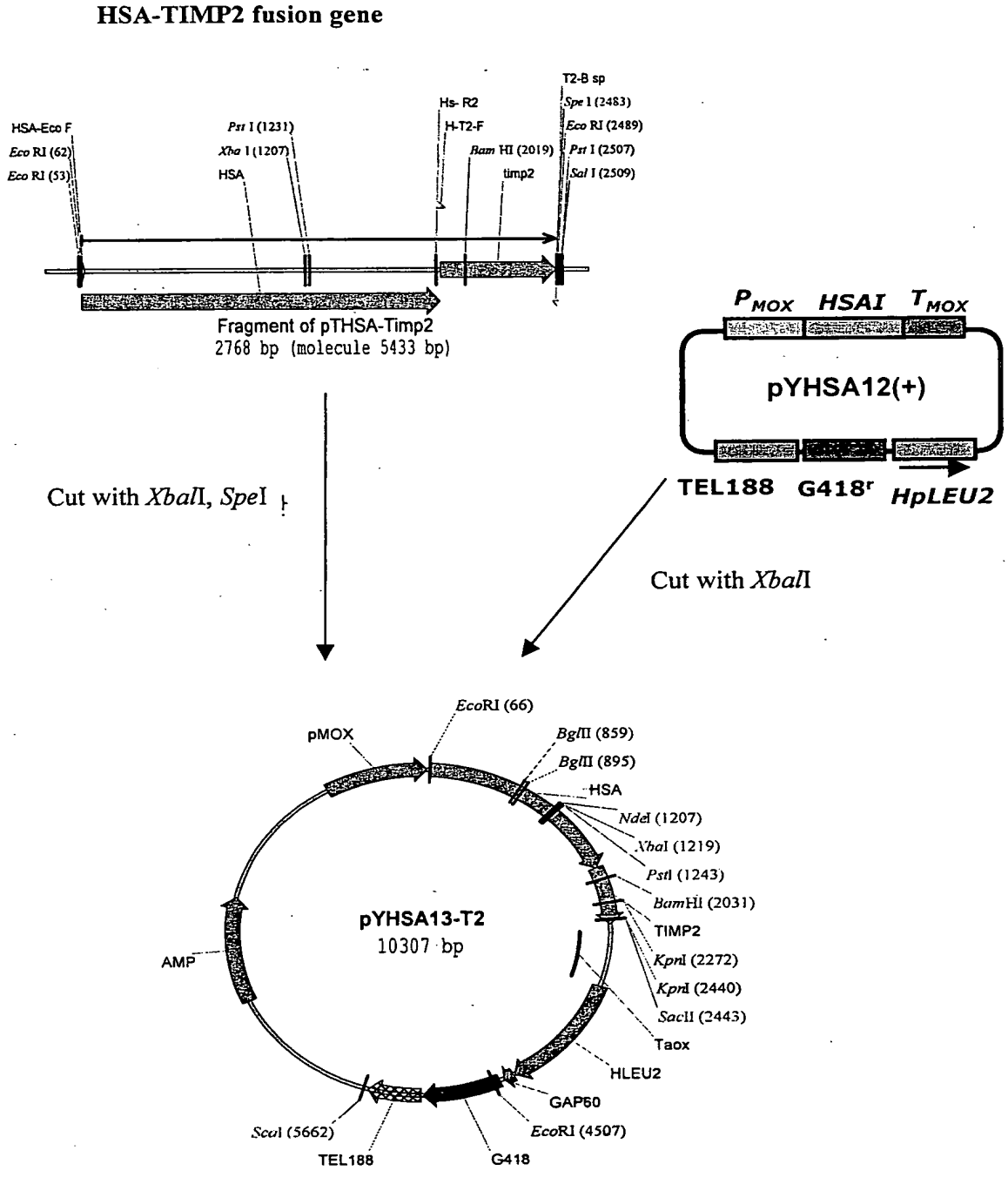

HSA-TIMP2 fusion gene
HSA-Eco F
Eco RI (62)
Eco RI (53)
Pst I (1231)
Xba I (1207)
HSA
Hs- R2
H-T2-F
Bam HI (2019)
timp2
T2-B sp
Spe I (2483)
Eco RI (2489)
Pst I (2507)
Sal I (2509)
Fragment of pTHSA-Timp2
2768 bp (molecule 5433 bp)
Cut with XbaI, SpeI
P MOX HSAI T MOX
pYHSA12(+)
TEL188 G418r HpLEU2
Cut with XbaI
pMOX
EcoRI (66)
BglII (859)
BglII (895)
HSA
NdeI (1207)
XhaI (1219)
PstI (1243)
BamHI (2031)
TIMP2
KpnI (2272)
KpnI (2440)
SacII (2443)
Taox
HLEU2
GAP60
EcoRI (4507)
G418
TEL188
ScaI (5662)
AMP
pYHSA13-T2
10307 bp

FIG. 10